EFFICACY OF ALBENDAZOLE IN REDUCING FECAL EGG COUNTS OF SOIL TRANSMITTED HELMINTHS IN SCHOOL CHILDREN

A Dissertation submitted to the Tamil Nadu Dr. M.G.R. Medical University, Chennai for the M.D. Degree in Microbiology.

April 2011

CERTIFICATE

This is to certify that the thesis entitled **"Efficacy of Albendazole in Reducing Fecal Egg Counts of Soil Transmitted Helminths in School Children"** is the bonafide work done by Dr. Vipin Sam Alexander in part fulfillment of the rules and regulations for M.D. Branch IV (Microbiology) examination of the Tamil Nadu Dr. M.G.R. Medical University, Chennai to be held in April 2011. This work was carried out under the guidance of Dr. Gagandeep Kang, Professor of Microbiology.

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ACKNOWLEDGEMENTS

I would like to elucidate a deep sense of gratitude to my guide, Dr. Gagandeep Kang for her guidance, encouragement, patience and support throughout the dissertation.

I would also take this opportunity to thank Dr. Mary Mathews, the Head of the department of Microbiology, CMC, Vellore for her advice and encouragement.

I would also like to thank Dr. Sitara Swarna Rao Ajjampur for her help during the various stages of the study.

I am grateful to the staff of Wellcome Microbiology Laboratory, especially Mrs. Sheela, Mrs. Selvi, Mr. Umar and Mr. Karthick for their immense help in the various technical aspects of the study.

I acknowledge Mr. Solomon from the department of Biostatistics for helping me with the statistical analysis of the results.

I am very thankful to my parents and friends for their love, moral support and encouragement.

Last, but not the least, I thank God for giving me the strength to complete my dissertation successfully.

CONTENTS

INTRODUCTION

Soil transmitted helminths (STH) are a group of intestinal nematodes whose infective forms undergo development in the soil, and cause infection in humans when they come in contact with the contaminated soil. *Ascaris lumbricoides, Trichuris trichiura* and hookworms are the most important and the most prevalent soil transmitted helminths. Infection with these worms is acquired by ingestion of eggs (*A. lumbricoides* and *T. trichiura)* from contaminated soil or by penetration of the skin by larvae in the soil (hookworms). They affect over 2 billion people in the world, majority of them residing in the tropical and subtropical regions of the world. STH infections are endemic in Africa, South-East Asia, Indian subcontinent, China, and Central and South America (de Silva, Brooker et al. 2003). In India, the prevalence of the three STH varies widely from 0 to 91% (Kaur, Rawat et al. 2002; Naish, McCarthy et al. 2004) .

STH infections are associated with low mortality, however, morbidity associated with them are debilitating, particularly in children who harbor heavy worm burdens. School aged children bear the brunt of STH infections, in whom they cause malnutrition, growth retardation, cognitive impairment and iron deficiency anemia (Drake, Jukes et al. 2000; Stephenson, Latham et al. 2000; Crompton and Nesheim 2002). However, these clinical effects have been shown to be reversible following treatment with anthelminthics (Stephenson, Latham et al. 1989). Preventive chemotherapy is one of the most widely used strategies for the control of STH infections. People at high risk are treated with benzimidazole anthelminthics (albendazole and mebendazole) without prior diagnosis (Hotez, Brindley et al. 2008).

Control programmes for STH using mass chemotherapy have been going on for a long time in different endemic regions of the world. The problem of anthelminthic resistance is common in

animals where anthelminthic drugs are being used widely and frequently (Geerts and Gryseels 2001). A similar problem is feared to occur in humans as evidenced by some studies in which a reduced efficacy of anthelminthic in the treatment of STH was documented (De Clercq, Sacko et al. 1997; Flohr, Tuyen et al. 2007). As there are very few available anthelminthics, it is important to monitor the drug efficacy in these programmes to detect the development of drug resistance. In various large scale treatment programmes, drug efficacy studies are being conducted infrequently, without a coherent long-term strategy and without a standardized monitoring protocol. During a WHO-World Bank meeting in 2007 it was decided that monitoring the efficacy of anthelminthics should be a key issue, and a Working Group was established to make recommendations for "Monitoring of drug efficacy in large scale treatment programmes for human STH". It was decided to adapt fecal egg count reduction test (FECRT) in human studies and integrate it into the monitoring of STH control programmes. FECRT is a widely used test in veterinary parasitology to detect anthelminthic resistance in which fecal egg counts are compared before and after treatment with an anthelminthic (Geerts and Gryseels 2000). This test has been standardized for detection of resistance in nematodes of veterinary importance (Coles, Bauer et al. 1992). WHO has also proposed to use a standard quantitative technique for determining fecal egg counts which is sensitive, simple and easy to perform in field conditions. In most of the drug efficacy studies, Kato Katz is the most commonly used quantitative technique, but this technique is not appropriate for detection of hookworm ova as it is too cumbersome to be used in field conditions. McMaster's method is a simple method which has been widely used in veterinary parasitology to determine fecal egg counts. However, McMaster's method has been used infrequently for determining egg counts in humans.

In this study, we assessed the efficacy of single dose 400 mg albendazole in terms of cure rates and egg reduction rates on schoolchildren infected with STH (Hookworms, *A. lumbricoides* and *T. trichiura*) from two districts–Vellore (an area whose residents has been receiving MDA with Albendazole for several years) and Thiruvannamalai (an area where MDA with Albendazole was started only recently). For this purpose, stool samples in school children from the two districts were screened for the presence of STH.

AIMS AND OBJECTIVES

Aim

- To assess the efficacy of albendazole in the treatment of Soil transmitted helminths (STH) in school children, living in areas that were or were not part of an annual mass drug administration with albendazole.

Objectives

1. To assess the change in fecal egg counts (FEC) in school age children, between 10 and 14 days following treatment with a single dose 400 mg of albendazole.
2. To monitor efficacy by determining the Cure Rate (CR) and Egg Reduction Rate (ERR).
3. To evaluate the suitability of the fecal egg count reduction test (FECRT) as a standard tool to monitor efficacy i.e. to develop a robust analytical approach that accounts for possible confounding factors.
4. To compare the relative performance of the Kato Katz and the McMaster egg counting technique.

REVIEW OF LITERATURE

Soil transmitted helminths (STH) are a group of parasitic nematode worms causing human infection through contact with parasite eggs or larvae that thrive in the warm and moist soil of the world's tropical and subtropical countries (Bethony, Brooker et al. 2006). Roundworm (*Ascaris lumbricoides*), whipworm (*Trichuris trichiura*), and hookworms (*Necator americanus* and *Ancylostoma duodenale)* are the most prevalent worms among the STHs. Although there are other STHs such as *Strongyloides stercoralis,* they are often under diagnosed as the most commonly used diagnostic methods have a low sensitivity in detecting the larvae in stool and special techniques are required for detection. Another enteric nematode, *Enterobius vermicularis* has a worldwide distribution, being the most common parasitic infection in temperate regions apart from being also co distributed with STHs in the tropical areas. The eggs of *E. vermicularis* are laid in the perianal area of the host and do not undergo development in soil, and hence it is not considered a classical STH. Schistosomes are frequently co-endemic with STH in some African countries. They are mainly transmitted by contact with water contaminated with the infected intermediate host.

MORPHOLOGY AND TAXONOMY

Ascaris lumbricoides

A. lumbricoides belongs to the class *Chromadorea*, order *Ascaridida*, superfamily *Ascaridoidea* and family *Ascarididae* (Anderson, Chabaud et al. 1974).

Eggs - The eggs of *A. lumbricoides* are seen in two forms in human stool samples: unfertilized and fertilized. The eggs appear yellowish brown in color, a feature called bile staining. The

fertilized eggs are spherical or ovoid in shape and measure 55 to 75 by 35 to 50 μm. The egg contains a large unsegmented ovum which almost fills the egg except for a small crescentic space at the poles (Todd 1948). The ovum is surrounded by a three layered shell, an inner lipid layer, a middle layer of chitin-protein and an outer vitelline layer. The inner layer contains a lipoprotein known as ascaroside, which helps the egg to survive the effects of several different chemicals. An uneven deposit of mucopolysaccharide (the cortical layer) can be seen on its outer surface, which helps in adhesion of eggs to different surfaces (Kagei 1983). Some eggs may not have this deposit and these are known as decorticated eggs. Unfertilized eggs are more elongated, measuring 85 to 95 by 43 to 47 μm, and do not contain the ovum.

Larvae - The first-stage larva of *A. lumbricoides* molts inside the eggshell and forms the second-stage larva. The second-stage larva has a typical filariform appearance and measures about 250 × 14 μm (Nichols 1956). It grows further to about 560 × 28 μm in length before undergoing a second molt in the lungs. After the second molt, the larva grows to a size of 1 mm. After the fourth (final) molt in the small intestine, it grows rapidly.

Adult worms - Adult *Ascaris* are the largest nematode worms. The adult worms preferably reside in the jejunum (Makidono 1956). Adult males and females appear pinkish cream in color, with males measuring 15-31 cm and females measuring 20- 35 cm in length. The mouth of the adult worms has three lips with teeth present in all the lips. The male worm has a curved posterior tail while the female worm has a straight posterior end.

Trichuris trichiura

T. trichiura belongs to the class *Adenophorea*, order *Enoplida*, family *Trichuridae* and genus *Trichuris* (Anderson, Chabaud et al. 1974).

Eggs - The eggs of *T. trichiura* are barrel shaped with a characteristic plug at each end. They appear yellowish brown in stool samples and measure 50–55 × 20–24 μm.

Larvae - On hatching in the intestine, the second-stage larva measures about 260 × 15 μm in length. It burrows into the intestinal mucosa where it undergoes a series of four molts to develop into adult worms.

Adults- Adult worms have a characteristic shape from which the common name whipworm is derived. The anterior end of the worms is long and thin bearing the resemblance of a whip while the posterior end is short and thick resembling the handle of a whip. The worms appear white in colour. The male worms are 30–45 mm long, while the females are longer measuring 30–50 mm in length. The posterior end of the male worms is coiled.

Hookworms -*Ancylostoma duodenale and Necator americanus*

Both the hookworms belong to the order *Strongylida* and the family *Ancylostomatidae* (Anderson, Chabaud et al. 1974).

Eggs- The eggs of the two species of hookworm are indistinguishable. They are oval in shape and measure about 60 × 75 × 36−40 μm (WHO 1994). They have a thin hyaline shell which encloses the developing embryo. The embryo is usually in a four or eight cell stage when seen in freshly passed feces.

Larva - Rhabditiform larva, which hatches from the egg, measures about 200 μm in length. This is a free living form which feeds on organic matter. It molts to form the non-infective second stage larva (L_2) measuring about 500 μm in length, which in turn molts to form the infective third-stage larva. The infective form is 500-700 μm long, those of *Ancylostoma* being generally longer than those of *Necator* (WHO 1981).

Adult- For both species, male worms (5–11 mm) are shorter than females (9–13 mm). *N. americanus* is generally shorter and more slender than *A. duodenale*. The term 'hookworm' is derived from the curve in the anterior tip of *N. americanus.* Proof of identity of a particular species is obtained by comparing the morphologies of the buccal capsule. The mouth opening of *A. duodenale* has two pairs of teeth, while that of *N. americanus* has two cutting plates.

LIFE CYCLE OF THE SOIL TRANSMITTED HELMINTHS

Ascaris lumbricoides

Eggs swallowed by humans hatch in the small intestine and release larvae which penetrate the intestinal wall and migrate via the venous blood through the liver to the heart, reaching the lungs. Following a second molting in the lung, the eggs break into the alveoli, ascend the tracheobronchial tree and are then swallowed to return to the intestines where they develop into mature worms. Adult female worms start producing eggs about 2-3 months following ingestion of eggs. Female worms produce about 200,000 eggs per day (Sinniah 1982). The adult worms are known to survive for a period of 1-2 years.

Eggs are passed in the feces in the unembryonated stage. In the presence of adequate moisture, shade and oxygen, the eggs embryonate to produce second stage larvae.

Trichuris trichiura

Humans develop infection with whipworm when they ingest embryonated eggs. The ingested eggs hatch in the small intestine to give rise to larvae which then pass into the large intestine. In the large intestine, these larvae undergo a series of molts mainly within the mucosal crypts of the cecum and mature into adults within about 3 months. After mating, the female worm produces

about 7000 to 20,000 eggs each day, which are shed in the feces. Under optimal conditions in the soil, eggs embryonate and become infective within 2 to 4 weeks.

Hookworms

The larvae enter the venous circulation to reach the lung capillaries from where they penetrate the alveolar walls, and make their way up the trachea to be swallowed and carried to their habitat in the small intestine. In the duodenum or jejunum, third stage larvae molt to become fourth stage larvae which finally develop into adult worms. Gravid females start egg deposition 5 to 6 weeks after skin penetration.

Eggs which are passed in the feces are non infective. Under suitable conditions of humidity, temperature, and shade, the eggs hatch into rhabditiform larvae in 24- 48 hours.

CLINICAL FEATURES

Clinical manifestations of STH infections occur mainly in those who have moderate or high worm burdens, especially children. Lighter infections with STHs are usually asymptomatic.

Ascariasis

Most cases with *Ascaris* infections are asymptomatic. The symptoms mainly occur due to the larval migration phase or in heavy infestations with the adult worm. In the early stages, the larval migration through the lung may elicit a hypersensitivity reaction resulting in nonproductive cough, chest discomfort, fever, and eosinophilia (Gelpi and Mustafa 1968).

The most severe consequences of ascariasis occur when a bolus of worms causes obstruction of small bowel, pancreatic, biliary ducts or appendix. Intestinal obstruction is the commonest complication and accounts for about 35% of cases with complications in the developing

countries (O'Hanley P 1995). It is more commonly seen in children, presumably due to the smaller circumference of their lumen, and is usually seen near the ileocaecal valve. It can be complicated by intussusceptions, volvulus, intestinal perforation, and gangrene of the bowel (Khuroo 1996).

The migration of adult worm to the biliary tree results in biliary colic, cholecystitis, cholangitis, and hepatic abscesses. Pancreatic duct obstruction results in pancreatitis. Hepatopancreatic complications are more commonly seen in adults, especially in females.

Trichuriasis

T. trichiura mainly resides in the caecum with its anterior end embedded in the colonic epithelium, while the posterior end extrudes into the lumen. The worms cause colitis by direct epithelial damage, as well as by inducing host inflammatory cells particularly macrophages as indicated by high levels of TNF-α in the colonic mucosa (MacDonald, Spencer et al. 1994).

Trichuris Dysentery syndrome (TDS) is a severe form of colitis seen in heavy infections and is characterized by chronic dysentery, rectal prolapse, anaemia, poor growth and clubbing of the fingers. The stunting seen in TDS has been hypothesized to be due to chronic inflammatory response, concomitant decreases in plasma insulin-like growth factor-1 (IGF-1), increases in TNF-α in the lamina propria of the colonic mucosa and peripheral blood, and a decrease in collagen synthesis (Stephenson, Holland et al. 2000). Several studies have shown that there is improvement in growth following treatment (Stephenson, Latham et al. 1989; Stephenson, Latham et al. 1993; Simeon, Grantham-McGregor et al. 1995). Chronic heavy infections results in growth retardation, intellectual and cognitive impairments (Nokes, Grantham-McGregor et al.

1992; Drake, Jukes et al. 2000). The blood loss that can occur in *T. trichiura* infection is likely to contribute to anaemia.

Hookworm infestation

At the site of entry of the hookworm larvae, particularly on the hands and feet, an erythematous papulovesicular rash may appear. These lesions are highly pruritic and are known as ground itch. These lesions are commonly seen in areas of high transmission where repeated exposures to the larvae occur (Hotez, Brooker et al. 2004). The migration of the larvae through the lungs can result in a disease resembling Loeffler's pneumonia, which is characterized by cough, sore throat, fever and eosinophilia in the lung (Miller 1979). The entry of the larvae of *A. duodenale* through the oral route may result in Wakana disease, which is characterized by nausea, vomiting, pharyngeal irritation, cough, dyspnea and hoarseness (Harada 1962).

The chief pathology associated with hookworm infection is intestinal blood loss. Blood loss occurs when the adult worms attach to the intestinal mucosa by their cutting apparatus, subsequently causing lacerations to capillaries and arterioles in the intestinal mucosa and submucosa (Hotez, Brooker et al. 2004). A continuous flow of blood is maintained by the release of anticoagulants (factor Xa and VIIa/TF inhibitors) and anti-platelet factors (Stanssens, Bergum et al. 1996; Del Valle, Jones et al. 2003). The hookworms change their position of feeding every 4-6 hours, but bleeding from the previous site continues due to the residual anticoagulant activity. The host hemoglobin is acted upon by hemoglobinases present in the brush border membrane of the parasite's alimentary canal.

Each adult hookworm causes up to 0.2 ml of blood loss per day (Roche and Layrisse 1966). When the blood loss exceeds the body iron stores, it results in iron deficiency anemia. The

tendency to develop anemia also depends on other factors like the type of hookworm species, the intensity and the duration of infection and dietary iron intake. *A. duodenale* causes greater blood loss than does infection with *N. americanus* (Albonico, Stoltzfus et al. 1998).

TRANSMISSION

Humans acquire roundworm and whipworm infection by the ingestion of embryonated eggs through faecal contamination. As the eggs of *A. lumbricoides* are sticky, they may adhere to fruit, vegetables, soil, dust particles, utensils, furniture, door handles, currency notes, flies and cockroaches (Kagei 1983; Tatfeng, Usuanlele et al. 2005). In endemic areas, eggs may become airborne, resulting in a risk of the embryonated eggs being inhaled and swallowed (Kroeger, Schulz et al. 1992). The infective third stage larvae or the filariform larvae of hookworms penetrate the skin of human beings when they come in contact with contaminated soil. Less commonly, infection with *A. duodenale* can also be acquired when the filariform larvae are ingested. STH parasites do not multiply in the host and along with the other helminths, follow common transmission dynamics. The morbidity of STH infections is associated with the intensity of infection, rather than the presence or absence of infection. The higher the intensity of infection, the greater will be the associated morbidity (Bundy and de Silva 1998). The nonlinear relationship between prevalence and intensity is mainly due to over-dispersed or aggregated distribution of the worm burdens. Over-dispersed distribution of worms is one in which in a particular endemic area, most individuals harbor few worms in their intestines while a few hosts harbor disproportionately large worm burdens (Anderson and May 1985). Such heavily infected individuals are at a higher risk of disease and also act as the major source of environmental contamination.

EPIDEMIOLOGY

STH are the most prevalent parasites, infecting over 2 billion people worldwide (Bethony, Brooker et al. 2006). *A. lumbricoides* infects about 807 to 1,221 million people, *T. trichiura* 604 to 795 million people and hookworms 576-740 million people (Bethony, Brooker et al. 2006). They are highly prevalent in the tropical and subtropical regions of the world, particularly in poverty stricken areas of the developing world.

The greatest number of STH infections occur in sub Saharan Africa (Brooker, Clements et al. 2006). The majority of *Ascaris* infections occur in China and Southeast Asia, and in western and central Africa. The highest prevalence of *Trichuris* infections is seen in central Africa, southern India, and Southeast Asia whereas hookworm infections are highly prevalent in sub-Saharan Africa, South China and Southeast Asia (de Silva, Brooker et al. 2003). In India, surveys conducted by the National Centre for Disease Control, Delhi, have estimated that *A. lumbricoides, T. trichiura* and hookworms affect 140, 73 and 71 million, respectively (de Silva 2005).

The mortality estimated to be associated with STH infections varies from 12000 to 135000 per year (WHO 2002). But these figures do not reflect the burden associated with these infections. A true estimate of the health burden would be provided by disability adjusted life years or DALYs, which are estimates based on cost and quality of life, deployed by the WHO. The annual DALY cost of STH has been estimated to be about 4.7 million years (Bethony, Brooker et al. 2006).

The groups which are at highest risk of morbidity and are most vulnerable to these infections are pre-school and school-aged children, adolescent girls and pregnant women (Montresor, Stoltzfus et al. 2002; Goodman, Haji et al. 2007). It has been estimated that *A. lumbricoides*, hookworm and *T. trichiura* infect 320 million, 239 million and 233 million school-age children worldwide,

respectively (Jardim-Botelho, Raff et al. 2008). Table 1 shows the prevalence of STH in school going children in different parts of the world.

For *A. lumbricoides* and *T. trichiura,* the prevalence of intensity gradually increases with age, rising to reach a peak around 5-10 years of age and declines thereafter (Bundy and de Silva 1998). However, the age-intensity profiles for hookworms varies, with some studies showing a convex age-intensity profile (Udonsi 1984; Behnke, De Clercq et al. 2000), while other studies showed that the prevalence of intensity continues to rise throughout life and is highest among the elderly (Gandhi, Jizhang et al. 2001; Bethony, Chen et al. 2002).

Table 1: Prevalence studies of STH in school children in different parts of the world

South America

COUNTRY	**PREVALENCE (%)**			**REFERENCES**
	A. lumbricoides	***T. trichiura***	**Hookworms**	
Brazil	4.8-31.8	4.9-38.6	1.8-9.9	(Maia, Fausto et al. 2009), (Brito, Barreto et al. 2006), (Prado, Barreto et al. 2001), (Rocha, Silva et al. 2000), (Tsuyuoka, Bailey et al. 1999), (Cury, Salles et al. 1994)
Nicaragua	20.7	34.7	1.4	(Rosewell, Robleto et al. 2010)
Cuba	3-40.5	8-35.5	5.5-9	(Escobedo, Canete et al. 2007), (Wordemann, Polman et al. 2006)
Mexico	3-14.5	6- 16	-	(Quihui, Valencia et al. 2006), (Diaz, Mondragon et al. 2003)

Asia

COUNTRY	PREVALENCE (%)			REFERENCES
	A. lumbricoides	***T. trichiura***	**Hookworms**	
Thailand	0-21.7	0.05-66.9	0.3-80	(Anantaphruti, Waikagul et al. 2004), (Waikagul, Krudsood et al. 2002), (Muennoo 2003), (Piangjai, Sukontason et al. 2003), (Tomono, Anantaphruti et al. 2003)
Malaysia	7.9-67.8	38.9-98.2	10.8-28.8	(Al-Mekhlafi, Atiya et al. 2007), (Al-Mekhlafi, Azlin et al. 2006), (Zulkifli, Anuar et al. 2000), (Norhayati, Oothuman et al. 1998), (Norhayati, Zainudin et al. 1997), (Rajeswari, Sinniah et al. 1994)
Vietnam	0.5-34	1.9-67	3-23	(Le Hung, de Vries et al. 2005), (Uga, Hoa et al. 2005)
Cambodia	26.3-40	0.4-17	5- 65	(Chhakda, Muth et al. 2006), (Sinuon, Anantaphruti et al. 2003), (Lee, Bae et al. 2002)
Lao DPR	48.4-67.14	17.49-43.8	12.8-37.5	(Hohmann, Panzer et al. 2001), (Kobayashi, Vannachone et al. 1996)
Indonesia	44	76	9	(Pegelow, Gross et al. 1997)
Myanmar	48.5	57	6.5	(Montresor, Zin et al. 2004)
Nepal	1.7-72.6	3-35.1	1.6-23.7	(Yong, Sim et al. 2000), (Rai, Hirai et al. 2004), (Sharma, Rai et al. 2004), (Shrestha, Rai et al. 2007)
Bangladesh	68	56	53	(Martin, Keymer et al. 1983)

Africa

COUNTRY	PREVALENCE (%)			REFERENCES
	A. lumbricoides	*T. trichiura*	Hookworms	
Tanzania	0-79	0-96	11.9-96	(Mazigo, Waihenya et al. 2010), (Knopp, Mohammed et al. 2009), (Massa, Magnussen et al. 2009), (Knopp, Mohammed et al. 2008), (Albonico, Bickle et al. 2003), (Albonico, Ramsan et al. 2002), (Albonico, Stoltzfus et al. 1999), (Albonico, Chwaya et al. 1997), (Marti, Haji et al. 1996)
Nigeria	7.9-88.5	2.2-84.5	0.7-32.4	(Adeoye, Osayemi et al. 2007), (Agbolade, Agu et al. 2007), (Ihesiulor, Emokpae et al. 2007), (Egwunyenga., Andy et al. 2005), (Nwaorgu, Okeibunor et al. 1998), (Holland, Asaolu et al. 1989)
South Africa	19.4-89.2	50.6-83.6	0.08-83.2	(Appleton, Mosala et al. 2009), (Adams, Markus et al. 2005), (Saathoff, Olsen et al. 2004), (Taylor, Jinabhai et al. 2001)
Kenya	14.2-50	7-42.6	30-42.5	(Handzel, Karanja et al. 2003), (Riesel, Ochieng et al. 2010), (Geissler, Mwaniki et al. 1998)
Cameroon	26.4-85	31-95	1.4	(Tchuem Tchuente, Behnke et al. 2003), (Nkengazong, Njiokou et al. 2010), (Ratard, Kouemeni et al. 1991)
Uganda	6.3-9.3	5-12.9	2.4-43.5	(Kabatereine, Tukahebwa et al. 2005), (Standley, Adriko et al. 2009)
Ethiopia	17.8	3.4	4.3	(Worku, Erko et al. 2009)

Environment

The environment plays an important role in the transmission and development of these parasites. Ova develop faster at higher humidity, with studies showing that ova of *A. lumbricoides* and *T. trichiura* do not embryonate at a humidity less than 50% (Otto 1929; Spindler 1929). Experimental data have shown that optimal development of hookworm larva occurs at 30º C and the ova of *A. lumbricoides* and *T. trichiura* embryonated well between 28^{o} C-32^{o} C, with poor development occurring at temperatures below 20^{o} C and temperatures above 40^{o} C (Beer 1976; Udonsi and Atata 1987; Smith and Schad 1989). However, field studies have shown that hookworm is significantly more prevalent than *A. lumbricoides* and *T. trichiura,* in areas where the land surface temperature is greater than 40^{o} C (Ratard, Kouemeni et al. 1991; Ratard, Kouemeni et al. 1992; Brooker, Beasley et al. 2002).

Epidemiology in India

STHs are prevalent throughout India as shown by different studies done in various parts of the country (Table 2). *A. lumbricoides* is the most prevalent STH among children in India. In North India, the prevalence of *Ascaris* in children ranges from 0.8% to 69.2% and that of *T. trichiura* ranges from 2.4% to 30.8% (Kaur, Rawat et al. 2002; Wani, Ahmad et al. 2007; Wani, Ahmad et al. 2008; Wani, Ahmad et al. 2008). The low prevalence of 0.8% and 2.4% of *Ascaris* and *T. trichiura* respectively was observed in a hospital based study (Kaur, Rawat et al. 2002). Kashmir is known to be an endemic area for ascariasis which is reflected by the high prevalence of 68.3% and 68.9% in school aged children. A prevalence of *A. lumbricoides* of about 68.1% was estimated in a study conducted in a population of 1061 children in the age group of 1.5 – 3.5 years residing in the slums of urban Lucknow (Awasthi and Pandey 1997).

In South India, various studies have shown the prevalence of *A. lumbricoides*, hookworm and *T. trichiura* in children to range from 0.5% to 91.3%, 0% to 51% and 2.01% to 72% respectively. High prevalence of 92.6% was observed in a study conducted in school age children in a fishing village near Visakhapatnam (Naish, McCarthy et al. 2004). The urban rural dichotomy of STH distribution was observed in a study conducted by Fernandez et al (Fernandez, Verghese et al. 2002).

The National Centre for Disease Control (NCDC, formerly NICD) has been conducting surveys in school children in different ecological zones of India. A survey was conducted in eight different ecologically homogenous zones in school aged children in the plains (Bhiwani in Haryana State, Pune in Maharashtra and Chitradurga in Karnataka), on the western coast (Calicut and Allepey in Kerala), in the deserts of Rajasthan (Alwar and Jodhpur) and Gangtok in the hills of Sikkim. The prevalence of STH was high in the hilly and coastal areas ranging from 34% to 36%. The highest prevalence of ascariasis was found in urban Chitradurga (39.7%), in rural Gangtok (30.8%) and in Calicut (25.7%). The prevalence of ascariasis was very low in Haryana and Rajasthan, which are both in the northwest of India. Trichuriasis was very low in most areas. The highest prevalence was seen in Calicut (25%) and Allepey (21.6%) in Kerala (Bora, Singh et al. 2001).

Table 2: Prevalence of soil transmitted helminths in children in India

SITE OF STUDY	N	SETTING	AGE (yrs)	PREVALENCE (%)			REFERENCES
				A. lumbricoides	Hookworm	*T. trichiura*	
Delhi	127	Hospital based	0-14	0.8	0	2.4	(Kaur, Rawat et al. 2002)
Lucknow	1040	Urban slums	1.5- 3.5	68.1	-	-	(Awasthi and Pandey 1997)
Kashmir	312	Rural	4-15	69.2	NA	30.8	(Wani, Ahmad et al. 2007)
Kashmir	2256	-	0- 15	68.3	-	27.9	(Wani, Ahmad et al. 2008)
Kashmir	382	-	5-15	23.8	-	15.2	(Wani, Ahmad et al. 2008)
Uttaranchal	257	School survey	9-10	28.8	5.1	1.9	(Bora, Meena et al. 2006)
Vizag	204	Rural	5-9	91	54	72	(Naish, McCarthy et al. 2004)
Chennai	125	Rural	school children	52.8	37.6	45.6	(Fernandez, Verghese et al. 2002)
Chennai	199	Urban	school children	0.5	0	2.01	(Fernandez, Verghese et al. 2002)
Tamil Nadu	342	Urban	<1->5	20.8	11.4	NA	(Ganga and Ravichandran 1995)
Vizag	217	Rural	7-13	75	9	66	(Paul and Gnanamani 1998)
Andhra Pradesh	151	Rural	6-12	91.3	45	70.8	(Mani, Rao et al. 1993)
Tamil Nadu	646	Rural	school children	53.9	12.4	5.7	(Mani, Rajendran et al. 2002)

DIAGNOSIS

Direct wet preparation is a presumptive test to identify the presence of helminthic eggs in feces. Saline wet mount is mainly used to detect helminthic eggs. Direct wet mount provides a rough estimate of the eggs present. It cannot be used to detect eggs in preserved samples or in samples containing minimal amount of eggs. If the mount is too thick, the eggs may be stained poorly and can be difficult to distinguish from background debris.

Concentration techniques help in detecting small number of parasites which are missed by direct wet preparation of the stool. These techniques separate out the parasites from the faecal material by differences in their specific gravity. These techniques are usually of two types, sedimentation and flotation techniques. In sedimentation techniques, eggs can be recovered at the bottom by using either gravity or centrifugation. It is easier to perform, causes less distortion of the eggs and is least subject to technical error (Garcia 2007). However, there can be excess of faecal debris in the sediment leading to difficulty in recovering the eggs. In flotation techniques, liquids with high specific gravity are used and the eggs can be recovered at the top of the fluid. Unfertilized eggs of *Ascaris* and larvae of *Strongyloides* do not float in the solution. High specific gravity of the fluid can cause distortion of the eggs.

Quantitation of eggs

Ascaris, *T. trichiura* and hookworm parasites are the only parasites for which it is reasonably possible to correlate egg production with worm burdens (Garcia 2007). A number of quantitative methods have been described. Primarily the FLOTAC and McMaster techniques are designed to be quantitative. The McMaster method is widely used in veterinary parasitology to determine fecal egg count. Kato Katz technique is most widely utilized method to determine fecal egg

counts in humans, but it is more appropriate for eggs of *Schistosoma mansoni* and less so for the detection of eggs of soil transmitted helminths. This technique is widely used in epidemiological surveys and is recommended by the WHO for monitoring helminth control programmes. Qualitative methods, such as zinc sulphate flotation or Ridley's formol-ether concentration have also been used for quantitation, but allow only semi quantitative determinations at best (Geerts and Gryseels 2000).

The sensitivity of a single Kato-Katz thick smear for hookworm diagnosis is low, particularly in cases of low infection intensities (Booth, Vounatsou et al. 2003). A study was done in Cˆote d'Ivoire, in which Kato—Katz and ether concentration methods were used to examine 3578 stool samples of schoolchildren. The sensitivities of the Kato—Katz technique and the ether concentration method for the detection of hookworms in this study was found to be 77.8% and 58.6%, respectively (Raso, Vounatsou et al. 2006). Another study conducted in Malawi, in which 988 stool samples were examined by Kato-Katz or ether concentration method showed prevalence of hookworms to be 27.3% and 19.3% with Kato-Katz and ether concentration respectively (Dacombe, Crampin et al. 2007). In Zanzibar, stool samples from 354 infants (aged 5—11 months) were examined by five different methods and the prevalence of any STH infection based on a duplicate Kato—Katz thick smear was 23.4%, whereas two ether concentration methods revealed prevalence of 18.7% and 19.8%. The sensitivities (Table 3) for detecting any STH infection by the Kato—Katz method and the two ether concentration methods were found to be 88.5%, 70.8% and 75.5% respectively (Goodman, Haji et al. 2007). The FLOTAC technique, has been described recently both for human and veterinary medicine, and its application for counting hook worm eggs described (Cringoli 2006; Utzinger, Rinaldi et al. 2008).

Table 3 – Prevalence and sensitivity of three methods for the detection of STH (adapted from (Goodman, Haji et al. 2007)

	Prevalence (%)				**Sensitivity (%)**			
	Any STH	***Ascaris***	***Trichuris***	**Hookworms**	**Any STH**	***Ascaris***	***Trichuris***	**Hookworms**
Kato Katz	23.4	10.2	22.6	6.1	88.5	77.1	96.5	81.5
Formol ethyl acetate sedimentation	18.7	8.3	12.1	6.3	70.8	62.5	51.8	77.8
Modified formol ethyl acetate sedimentation	19.8	9.1	14.6	6.9	75	68.8	62.4	66.7

TREATMENT

The WHO has recommended four anthelminthics - albendazole, mebendazole, levamisole and pyrantel pamoate (Utzinger and Keiser 2004). Albendazole and mebendazole belong to the benzimidazole group of anthelminthic drugs. Benzimidazoles act by binding to a cytoskeletal protein called β-tubulin, thus inhibiting its polymerization into microtubules (Lacey 1990). The absorptive functions of the parasitic intestinal cells are affected, thereby leading to decreased energy source. ATP formation does not occur as a result of which the parasites die. Albendazole is efficacious in the treatment of ascariasis and hookworm infections in a single dose. In a meta-analysis, different studies in which albendazole was given in a single dose of 400 mg showed a cure rate of 93.9% and egg reduction rates ranging from 86.5% to 100% for *A. lumbricoides,* and

a cure rate of 78.4% and egg reduction rates ranging from 64.2% to 100% for hookworms (Keiser and Utzinger 2008). However a single dose of albendazole has been known to be less efficacious for the treatment of *T. trichiura* infections with an overall cure rate of 78.4% and egg reduction rates ranging from 0% to 89.7%. Adams et.al conducted a study in which children infected with *T. trichiura* were randomized to 3 doses of albendazole (400, 800 or 1200 mg), each repeated 4 times. The cure rates were 23%, 56% and 67% and egg reduction rates were 96.8%, 99.3% and 99.7% for 400 mg, 800mg and 1200 mg doses respectively (Adams, Lombard et al. 2004). Thus, albendazole will be more efficacious in *T. trichiura* infections when given in higher/multiple doses. In another study done by Sirivichayakul et.al, 5 day or 7 day treatment with albendazole caused a significant reduction in cure rate and egg excretion in moderate and heavy intensity *T. trichiura* infections when compared to those receiving a three day course of albendazole (Sirivichayakul, Pojjaroen-Anant et al. 2003).

CONTROL

Chemotherapy, improved sanitation, hygiene and health education are the main strategies for control of STH. Preventive chemotherapy is the most widely used intervention in many STH control programmes. Periodic and repeated deworming with anthelminthics is considered as one of the most cost-effective strategies in the short term for controlling morbidity due to STH (Savioli, Bundy et al. 1992).

Preventive Chemotherapy

The main aim of chemotherapy is to reduce the morbidity in infected populations by decreasing the number of worms, thus preventing infection of healthy people in the same population. WHO

has advocated three categories for drug treatment-selective, targeted and universal. *Selective treatment* is one in which drugs are administered to individuals who are diagnosed with infection and this approach is followed where the prevalence of infections is <50% and of heavy infections <10%. In *targeted treatment* drugs are provided to a group of people with no prior diagnosis in areas where there is high prevalence of infection (>50%) but prevalence of heavy infections is less than 10%. *Universal treatment* is recommended for a population in which prevalence of heavy infections exceeds 10%. In this strategy, the whole community is treated irrespective of infection status. Because worms are most aggregated when prevalence is low, at a prevalence of <50% only a small proportion of infected people will have disease due to a moderate to heavy worm burden and will therefore benefit from treatment. When the prevalence of infection is >50%, the cost-effectiveness of treatment rises, because a larger proportion of the population have moderate to heavy worm burdens and are likely to be diseased. Periodic deworming i.e regular treatment of high risk groups with anthelminthics at regular intervals is known to keep the worm burden below the threshold associated with disease (Albonico, Montresor et al. 2006). Benzimidazole anthleminthics are the most commonly used drugs for STH control because of their broad spectrum of activity, low cost, high efficacy and ease of administration (Savioli, Stansfield et al. 2002; Brooker, Bethony et al. 2004).

Control programmes

Current STH control programmes using deworming mainly target school-age children as they are likely to be heavily infected with these worms, especially, *A. lumbricoides* and *T. trichiura*. They are also vulnerable to the detrimental effects of infections such as growth retardation (Adams, Stephenson et al. 1994) and decreased cognitive functions. Various studies have shown that

deworming has beneficial effects in school age children which include improvements in iron stores, growth and physical fitness (Stephenson, Latham et al. 1989; Stephenson, Latham et al. 1993) and cognitive performance. School based chemotherapy of children is considered to be a very cost effective method as schools have the necessary infrastructure. Properly trained teachers can be in charge of the drug delivery and of health education. The non-enrolled children can also be reached in an effective way if the enrolled children are asked to inform their parents, siblings and friends about the date of next deworming campaign (Montresor, Ramsan et al. 2001). In 2001, the World Health Assembly put forward a resolution (Resolution 54.19) to regularly treat at least 75% of all school-aged children and other high-risk groups with either albendazole or mebendazole alone or together with praziquantel by 2010 in endemic countries. In order to implement this strategy, the Partnership for Parasite Control (PPC) was launched. The PPC is composed of various UN agencies - United Nations Children's Fund (UNICEF), World Health Organization (WHO), World Food Programme (WFP), Office of the United Nations High Commissioner for Refugees (UNHCR), World Bank, research institutes, universities and various nongovernmental organizations. It has advised that deworming should be integrated with other control programmes for communicable diseases like malaria, tuberculosis, etc. It also proposed that deworming can also be integrated into other programmes like national immunization days, vitamin A supplementation programmes, feeding programmes, and water and sanitation initiatives. In Nepal, a scheme was conducted by the Nepalese government in partnership with WHO and the World Food Programme in which school children were given a nutritious meal during the school day and were also offered two doses of albendazole during the school year. A midterm survey was conducted which showed a decrease in prevalence of STH infection in the

treated children from 74% to 48% and a decrease in prevalence of heavy infections from 9% to 2% (Khanal and Walgate 2002).

In countries where STH is co-endemic with schistosomiasis, a combined approach to control both diseases has been followed. Schistosomiasis Control Initiative (SCI), a charitable organization is working with the governments of Burkina Faso, Mali, Niger, Tanzania (including Zanzibar), Uganda and Zambia where it is providing treatment with praziquantel and albendazole.

In Uganda, a national control program for schistosomiasis and STH was implemented from 2003 in which albendazole (400 mg) and praziquantel was given annually to schoolchildren in endemic areas and to adults in selected communities where local prevalence of *Schistosoma mansoni* in schoolchildren was high. In order to monitor the impact of the control programme, school children were examined from schools in three different areas. The prevalence of hookworm infection was reduced from 50.9% at baseline to 10% after two annual rounds of MDA with an overall reduction of 79.0% (Zhang, Koukounari et al. 2007).

Control programmes in India

As of now, no control programme exclusively targeting STH has been launched in India, but anthelminthic drugs are given as a part of various programmes like ICDS and the filariasis eradication programme. In Tamil Nadu state, as a part of filariasis control/elimination programme, Mass Drug Administration (MDA) with an annual single dose of Diethylcarbamazine (DEC) is being provided to 12 districts from 1998. However from 2001, the Tamil Nadu government decided to co-administer albendazole (ALB) with DEC in six districts (Mani, Rajendran et al. 2002). A study was conducted by Mani et.al to assess the impact of

MDA with ALB and DEC versus MDA with DEC alone on the prevalence of STH in village from two blocks in the Villupuram district of Tamil Nadu. In this study, two stool samples were collected (first sample before MDA and the second sample 3 weeks after MDA) from 646 children (321 in DEC and ALB intervention group and 325 in DEC alone group) in the age group of 9-10 years. It was observed that the prevalence of STH in the DEC and ALB group reduced from 60.44% at baseline to 15.56% at three weeks. The prevalence rates of *A. lumbricoides*, hookworms and *T. trichiura* reduced from 54.83%, 16.51% and 4.96% to 14.12%, 1.73% and 0.86% respectively. The odds of cure in the combination therapy arm for the STHs (5.7 times) were significantly higher than the odds of cure in the single-drug arm (Mani, Rajendran et al. 2002).

Another study was conducted by Mani et.al to assess the impact of two rounds of annual MDA with DEC and ALB on the prevalence of STH in the same villages where the above mentioned study was conducted. In this study, a cross-sectional survey on STHs was conducted before treatment, and then 11 months after the first and second MDA on school children (325–500) aged 9–10 years (4th and 5th standards) each in the DEC + ALB group and DEC alone group. The prevalence of STH decreased from 60.44% at baseline to 14.15% at two years after MDA with DEC and ALB (Mani, Rajendran et al. 2004).

SUMMARY

Soil transmitted helminths are one of the most prevalent parasites in the world, affecting almost half of the population living in the developing countries. These parasites share a general pattern in their life history and transmission. They mainly cause chronic effects such as growth retardation, protein energy malnutrition, iron deficiency anemia and impaired cognitive function.

These parasites are most prevalent and cause more severe infections in school aged children. Treatments with anthelminthics are shown to have beneficial effects in children such as improvement in growth and cognitive performance. Preventive chemotherapy is the commonest strategy used in the control of STH. Large scale control programmes using periodic mass drug administration of benzimidazole anthelminthics have been implemented in different endemic countries. Anthelminthic resistance is a frequent and widespread problem in livestock as a result of frequent periodic mass treatments. The problem of drug resistance is a major concern in human beings, particularly in endemic areas where mass drug administration is given and there is a need to monitor these programmes to detect drug resistance.

MATERIAL AND METHODS

Rationale for the study methods

As the widespread usage of anthelminthics in STH control programmes expands, anthelminthic resistance is becoming a problem in STHs of veterinary importance (Geerts and Gryseels 2000). With the use of anthelminthics in human control programmes, the development of a similar situation is a matter of concern. It is, therefore, very important to develop a standardized protocol to determine drug effectiveness in control programmes and detect drug resistance. Fecal egg count reduction test (FECRT) is a widely used test in veterinary parasitology to detect anthelminthic resistance in which fecal egg counts are compared before and after treatment with an anthelminthic (Coles, Bauer et al. 1992). In FECRT studies, animals from a particular farm are chosen and divided into two groups-one receiving treatment and the other receiving no treatment. Feces samples are collected twice–first on the day of treatment and the second sample between 10-14 days of treatment. The egg reduction rate (ERR) is calculated and resistance to that particular drug can be suspected if ERR is less than 95% and the lower 95% confidence interval is less than 90%. FECRT needs to be adapted in human studies to look for any drug resistance. For determining the egg counts, a quantitative technique which is simple, sensitive, practical and easy to use in field conditions should be considered. In FECRT studies in animals, McMaster method is the most commonly used technique to determine FEC. In human studies, the most widely used quantitative test has been the Kato Katz method. However, this test is very cumbersome to use under field conditions and is not appropriate for hookworm eggs. Also, this method has a low sensitivity in detecting low intensity infections.

Therefore, the World Health Organization Working Group proposed a study design for human FECRT studies based on treating a group of people and assessing fecal egg counts (FEC) before and after treatment, with the post treatment samples ideally be collected between 7 and 14 days. In this study, we worked with the WHO group and decided to assess the efficacy of albendazole by comparing the fecal egg counts pre-treatment and post-treatment in two STH infected groups of people, one group which has been receiving mass drug administration (MDA) with albendazole for a long time (Vellore district introduced MDA with diethylcarbamazine and albendazole in 2001) and another group not receiving mass drug administration (Thiruvannamalai district had not introduced MDA when our study started). We decided to include schoolchildren from Vellore and Thiruvannamalai district, for ease of access to study populations. A school survey was done initially in both areas in order to screen for positive cases of STH. Only those positive cases with a minimum FEC of 150 eggs per gram of each STH were included in the efficacy trial. Fecal egg counts were determined on stool samples collected on the day of treatment and 10-14 days post treatment. In this study, FEC was determined by using McMaster technique. As McMaster's method has been used very rarely in human studies, we decided to compare it with the commonly used Kato-Katz method and assess its sensitivity and feasibility.

Study site

The study was conducted in the Parasitology section of the Wellcome Trust Research Laboratory, Christian Medical College, Vellore.

Study design

The study was conducted in the following three phases:

1. *Pilot comparison of Quantification techniques*

A pilot study was conducted to compare Kato Katz method and McMaster method. This comparison was done on 100 stool samples which came to the parasitology laboratory for routine ova and cyst examination over a period of eight days in November 2008.

A study number was given to each sample and was processed within 24 hours after their receipt.

Each day, an average of 10 stool samples was processed. McMaster's and Kato-Katz method were performed on each stool sample. The McMasters method was performed in the same manner as for detecting egg count in the efficacy trial. However, two McMaster's chambers were examined for the pilot study. The fecal egg count was determined by counting the number of eggs under each grid of the chamber and multiplying it by 25. Kato Katz method was done as described later.

2. *School based survey*

The stool samples were collected from school children aged 6 to 14 years attending government and government aided schools in Vellore and Thiruvannamalai districts of Tamil Nadu state. The residents of Vellore district have been receiving albendazole as a part of National Filarial Control Programme, while Thiruvannamalai did not have the programme.

Study design: Non randomized parallel group trial

Study Duration: 1st December 2008 to 31st May, 2009

Collection of Stool samples

Schools were randomly selected from each district for carrying out the study after obtaining permission from the relevant government authorities. A detailed health education was given to children belonging to the selected schools regarding soil transmitted helminths, their transmission and prevention. Informed consent form was translated into the local language, Tamil and was provided to the selected children. The children were asked to show it to their parents or guardians to obtain their consent for participation in the study. The children who agreed to participate in the study were provided with a screw capped plastic container. A field worker went the next day to the child's home to collect the container. The stool samples, thus collected were sent to the laboratory within 3- 4 hours of collection. All children who provided a stool sample which was positive were treated with albendazole in a single dose of 400 mg under direct supervision.

The study subjects were selected based on the following inclusion and exclusion criteria

Inclusion Criteria:

1. Age 6-14 years
2. Ova of *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* seen on microscopic examination of stool.
3. Resident of Vellore district (for Albendazole MDA group) or resident of area where no government MDA program is administered (non-MDA group).
4. Willing to participate in the study and give informed consent and follow-up sample.

Exclusion Criteria:

1. Age <6 years, >14 years
2. No ova of *Ascaris lumbricoides*, hookworm and *Trichuris trichiura* seen in stool.
3. Not willing to participate.
4. Unable to give samples for follow up.

Laboratory Diagnosis

The stool samples were received in screw capped plastic containers. A lab identification number was given and the stool samples were examined on the same day. A wet preparation of the stool samples was made using saline and examined under the microscope for the presence of ova of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura.* Following the direct microscopy, a formol ether concentration technique was performed on the stool samples and examined by wet preparation.

3. *Efficacy trial*

On stool samples, which were positive for ova of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura*, McMaster's method was performed to determine the egg count. A single McMaster's chamber was used and number of eggs per gram of feces was determined by multiplying the total number of eggs under the two grids by 50. A second stool sample was collected only from those children whose pretreatment egg count was greater than 150 eggs per gram. The field workers provided screw capped containers to these children and the second stool sample was collected between 10 – 14 days after treatment. The second stool sample was re-examined by McMaster's method to determine the post treatment fecal egg count.

Coprological techniques

1. *Kato-Katz method*

The material required for this method comprised of a template with hole (diameter=9mm, thickness=1mm), screen, microscope slides and glycerol methylene blue solution (1 ml of 3% methylene blue in 100 ml of glycerol and 100 ml of distilled water). A small amount of stool sample was placed on a newspaper or scrap paper and the screen was placed on top of the sample to allow it to be filtered through the screen. The filtered sample was scraped with a spatula and placed in the hole of the template placed on the microscope slide until it was completely filled. The template was carefully removed so that a cylinder of the sample was left on the slide. The amount of feces on the microscope slide was measured for each sample. Two drops of the glycerol-methylene blue solution was added to the sample and mixed well. Another microscope slide was taken and pressed firmly against the first slide. The slides were incubated for twenty minutes at room temperature and examined under the microscope at 100x magnification. The fecal egg count was determined by multiplying the number of eggs found by the inverse of the weight.

2. *McMaster technique*

Requirements

1. McMasters counting chamber
2. Two plastic containers
3. Weighing machine
4. Measuring cylinder

5. Stirring device
6. Flotation fluid-Saturated salt solution

Procedure

1. 2 grams of feces was weighed and taken in one of the plastic containers.
2. 30 ml of the saturated salt solution was measured and added to the feces sample.
3. The feces sample was allowed to soak for a few minutes and then mixed or broken up with a spatula and then sieved through the tea strainer to withhold large debris.
4. McMasters counting camber was washed with water and then dried.
5. The feces solution was mixed thoroughly and a small amount of it was taken using the Pasteur pipette to fill both the grids of the chamber. Care was taken not to introduce any air bubbles inside the chamber.
6. The chamber was examined under 100x magnification and only the eggs present under the grid were counted.

Statistical analysis

All the data were entered into Microsoft Excel 2007 and analyzed using SPSS v16.0.

Pilot study

The pooled results by both McMaster and Kato Katz methods were taken as the diagnostic 'gold' standard. The prevalence of each STH infections and sensitivity of McMaster technique was determined. The agreement in qualitative test results between McMaster and Kato Katz methods was calculated by using Kappa statistics. The agreement in quantitative test results was estimated by the Spearman rank correlation coefficient. In addition, the Wilcoxon signed rank test was

assessed to test for differences in FEC between the techniques. For this end, a post-hoc Bonferroni pair-wise comparison procedure was performed and the level of significance was set at 0.016 (SAS 9.1.3, SAS Institute Inc.; Cary, NC, USA).

School survey

Prevalence of each STH was calculated using the following formula

Prevalence= (Number of children positive for each STH/Total number of examined children)*100

Efficacy trial

The following parameters were assessed

1. Cure rate (CR) was calculated by the following formula:

   CR= {[Number of children positive before treatment-Number of children positive after treatment]/ Number of children positive before treatment}*100

2. Egg reduction rate (ERR) was calculated by the following formula

   ERR= {[Sample mean T1-Sample meanT2]/Sample mean T1}*100

3. Mean reduction in FEC per individual was calculated as follows

   $$\left(\sum_{i=1}^{i=N} [T1_i - T2_i]\right) / N$$

   where T1 = pre-treatment FEC, T2 = post-treatment FEC, *i*= *i*th subject

4. Mean percentage reduction in FEC per individual was calculated as follows

   $$\left(\sum_{i=1}^{i=N} \{[T1_i - T2_i] / T1_i\}*100\right) / N$$

where T1 = pre-treatment FEC, T2 = post-treatment FEC, *i*= *i*th subject

Wilcoxon signed rank test was used to assess the differences between the pre-treatment and post-treatment FEC.

Ethical considerations

The study was approved by the Institutional Review Board of Christian Medical College, Vellore. A written informed consent was obtained from the parents of the children before collection of stool samples.

RESULTS

1. Pilot comparison of Kato-Katz and McMaster technique

A pilot comparison of the two techniques was conducted in the Parasitology section of the Wellcome Trust Research Laboratory over an 8 day period in November 2008. A total of 100 stool samples which were sent to the laboratory for routine ova and cysts examination were included in the study.

Of the 100 samples, McMaster's was positive in 43 and Kato-Katz in 48 for the detection of at least one STH in each sample. Overall, at least one STH was detected in 52 samples by either McMaster or Kato Katz technique. Hookworm was the most common STH (36%), followed by *A. lumbricoides* (12%) and *T. trichiura* (9%). The prevalence of STH detected by each of the techniques is shown in Table 4.

Table 4: Prevalence of each STH by McMaster and Kato-Katz method used in parallel on 100 samples

	Kato-Katz	**McMaster**
Hookworm	36%	33%
A. lumbricoides	10%	6%
T. trichiura	6%	7%

Sensitivity of McMaster when compared to Kato-Katz

Table 5 shows the performance of the McMaster technique in the detection of hookworms in comparison with the Kato-Katz method. The McMaster technique was able to detect 32 (88.9%)

out of the 36 positives detected by Kato-Katz method. McMaster technique also detected hookworm ova in one additional sample.

Table 5: Comparison of McMaster and Kato-Katz for Hookworms

		McMaster		Total
		Absent	Present	
Kato-Katz	Absent	63	1	64
	Present	4	32	36
Total		67	33	100

- Sensitivity of McMaster- 89%

Table 6 shows the performance of McMaster technique in the detection of *A. lumbricoides* in comparison with the Kato-Katz method. McMaster technique was able to detect only 4 (40%) out of the 10 positives detected by Kato-Katz method, but also detected ascariasis in two additional samples.

Table 6: Comparison of McMaster and Kato-Katz for *A. lumbricoides*

		McMaster		Total
		Absent	Present	
Kato-Katz	Absent	88	2	90
	Present	6	4	10
Total		94	6	100

- Sensitivity of McMaster- 50%

Table 7 shows the performance of the McMaster technique in the detection of *T. trichiura* in comparison with the Kato-Katz method. The McMaster technique detected only 4 (66.7%) out of the 6 positives detected by Kato-Katz method, but also identified three additional positive samples.

Table 7: Comparison of McMaster and Kato-Katz for *T. trichiura*

		McMaster		
		Absent	Present	Total
Kato-Katz	Absent	91	3	94
	Present	2	4	6
Total		93	7	100

- Sensitivity of McMaster- 78%

The strength of agreement between the two techniques was almost perfect for hookworm (K=0.89), while it was moderate for *A. lumbricoides* (K=0.46) and *T. trichiura* (K=0.59).

Quantitative test results

The agreement in quantitative test results was only considered for hookworms and not for *A. lumbricoides* and *T. trichiura.* There was a significant ($p < 0.001$) linear correlation (Rs) in FEC between the two techniques, ranging from 0.73 to 0.84 (Figure 1). However, the concordance plots showed a difference in level of agreement between the techniques. Other pair-

wise comparisons showed a slope smaller than 1, indicating that the technique in the x-axis is detecting more eggs than the technique in the y-axis. McMaster detected significantly more eggs compared to Kato-Katz technique ($p < 0.016$). These plots also indicate that techniques fail to detect low infection intensities. The amount of feces examined by the Kato-Katz ranged from 0.02 to 1.18g.

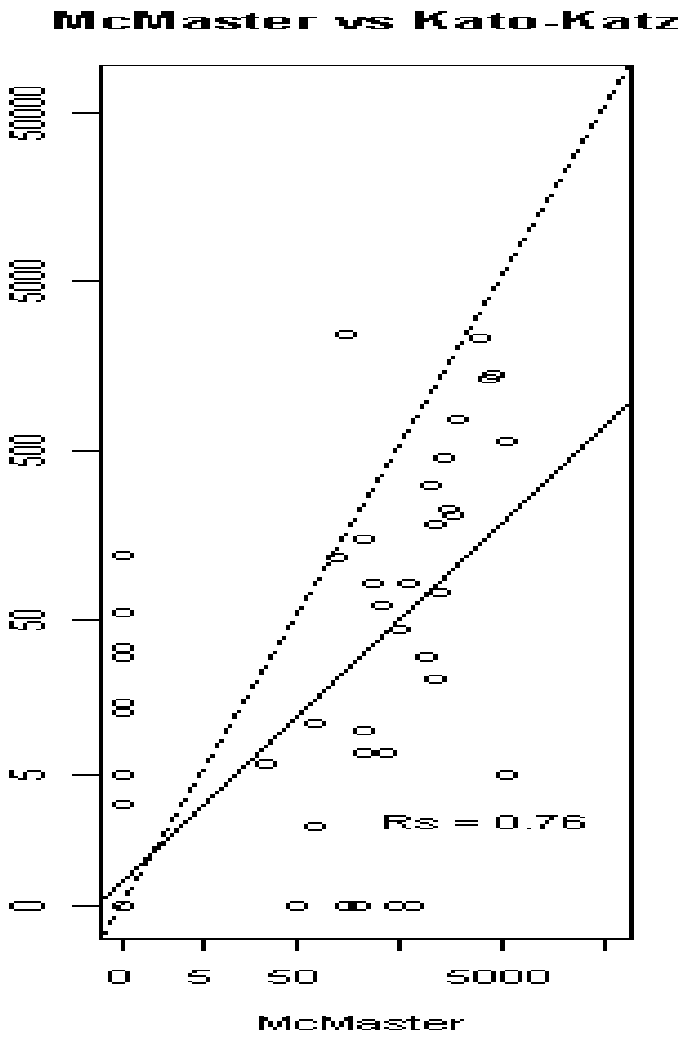

Figure 1: Pair-wise comparison of the McMaster and Kato-Katz technique based on FEC of hookworms

2. School survey for soil transmitted helminths

Between December 1, 2008 and May 31, 2009, stool samples from 2549 children were examined. Of these, 1161 were from Vellore district and 1388 from Thiruvannamalai district. Figure 2 shows a map of the 2 districts in which the school survey was carried out.

Figure 2: Map showing Vellore and Thiruvannamalai districts in which the school survey was conducted

Age distribution - All the children were in the age group of 6 to 14 years with the mean age being 11.2 years (SD=2.25). In Vellore, the mean age was 11.01 years (SD=2.3) and that for Thiruvannamalai was 11.4 years (SD=2.2).

Gender- Overall, 51% of the samples (n= 1300) were from males and 49% (n= 1249) from females. In Vellore, 48.4% (n=562) were males and 51.6% (n=599) were females, whereas in Thiruvannamalai, 53.2% (n=738) were males and 46.8% (n=650) were females. Figure 3 shows the distribution of males and females in both districts.

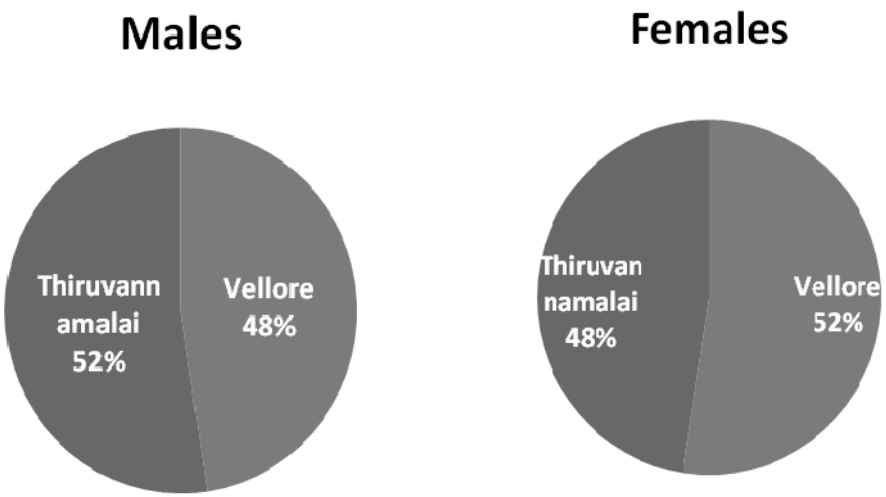

Figure 3: Gender distribution in samples population in Vellore and Thiruvannamalai districts

Baseline prevalence

Among the 2549 children, 161 (6.3%) were infected with at least one STH. Of these 161 children, 17 (10.6%) were infected with more than one STH. Mixed infections with both *A. lumbricoides* and *T. trichiura* were found in 15 children (9.3%), infections with hookworm and

T. trichiura occurred in 1 child (0.6%). One child was found to be infected with all the three STHs.

Table 8 shows the prevalence of each STH by district. The overall prevalence of STH in Vellore and Thiruvannamalai districts were 6.8% and 7.1% respectively. No statistically significant difference (p=0.8) was found between the overall prevalence of STH between the two districts. The prevalence of hookworms in Vellore and Thiruvannamalai were 2.1% and 6.6% respectively. The prevalence of *A. lumbricoides* was 2.8% and 0.2% in Vellore and Thiruvannamalai respectively. The prevalence of *T. trichiura* was 1.9% and 0.3% in Vellore and Thiruvannamalai respectively. A statistically significant difference (p<0.001) was observed between the prevalence of individual STH, with higher hookworm prevalence in Thiruvannamalai which was more rural, and higher prevalence of *Ascaris* and *Trichuris* in Vellore, which was more urban.

Table 8: Prevalence of STH in Vellore and Thiruvannamalai

	STH	***A. lumbricoides***	***T. trichiura***	**Hookworm**
Vellore (n= 1161)	6.8%	2.8%	1.9%	2.1%
Thiruvannamalai (n= 1388)	7.1%	0.2%	0.3%	6.6%
p-value	0.8	<0.001	<0.001	<0.001

Prevalence of individual STH in different age groups

The age wise prevalence of individual STH is shown in Figure 4. The prevalence of hookworm infection gradually increased with age, with 25% of the people infected with hookworms in the 14 year old age group. The prevalence of *Trichuris* and *Ascaris* infections followed a convex pattern with the prevalence gradually increasing from the 6 year olds to reach a maximum in the 8-10 year age group and then decreasing.

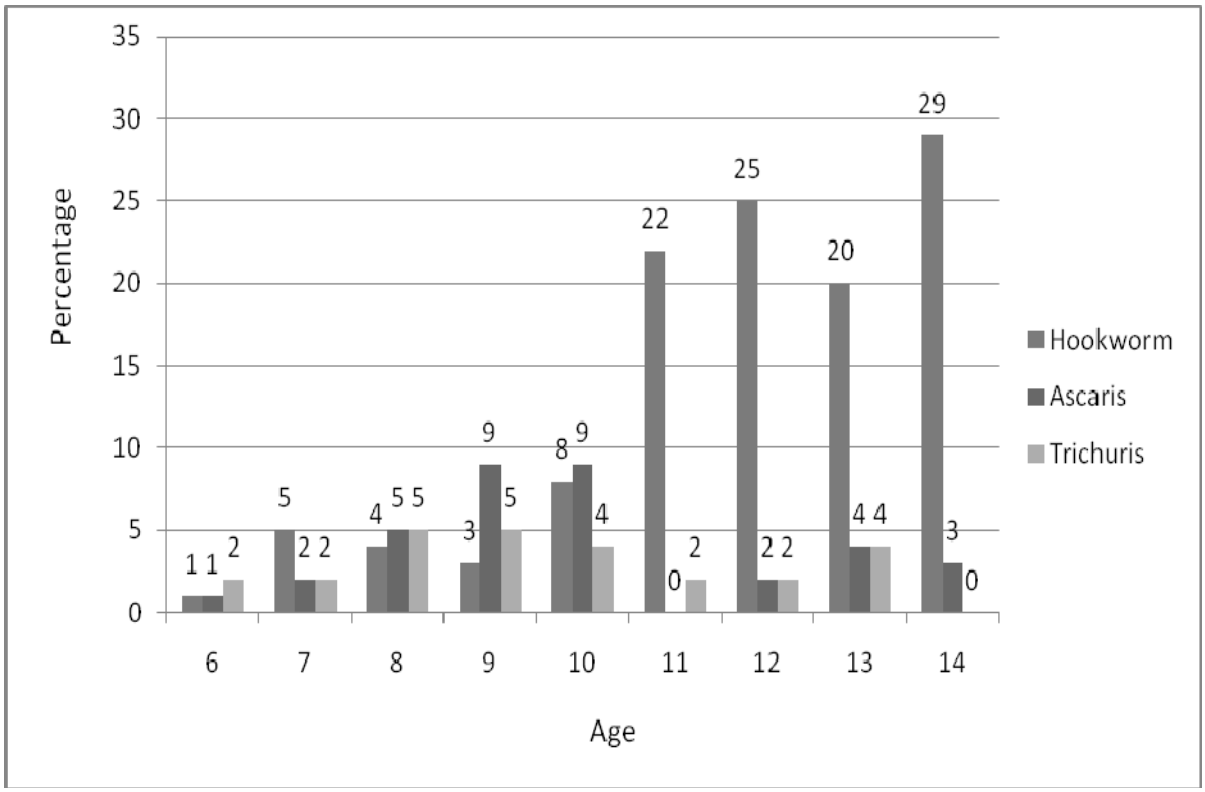

Figure 4: Prevalence of soil-transmitted helminths by age

Intensity of infection

Intensity of infection was measured in terms of eggs per gram of feces (epg). The intensity of infections was categorized into light, moderate or heavy infections (Table 9) based on the criteria laid down by WHO (WHO 2002). The majority of those infected with hookworm, *A. lumbricoides* or *T. trichiura* had light intensity infections. Light intensity infections were seen in 98.1%, 78.8% and 92.3% among those infected with hookworm, *A. lumbricoides* and *T. trichiura* respectively. Moderate intensity infections occurred in 1.9%, 21.2% and 7.7% of those infected with hookworm, *A. lumbricoides* and *T. trichiura* respectively. None of the children had heavy intensity infections. The mean eggs per gram were 333, 1733 and 325 for hookworms, *A. lumbricoides* and *T. trichiura,* respectively.

Table 9: Classes of Intensity for Soil transmitted helminths

	Light intensity infections	**Moderate-intensity infections**	**Heavy-intensity infections**
A. lumbricoides	1-4999 epg	5000-49999 epg	≥ 50000 epg
T. trichiura	1-999 epg	1000-9999 epg	≥ 10000 epg
Hookworms	1-1999 epg	2000-3999 epg	≥ 4000 epg

Relation between intensity of infection and age for individual STHs

The intensity of infection with hookworms gradually increased with age peaking in the 14 year olds. For *A. lumbricoides*, the intensity of infection gradually increased with age, peaking in the 10 year olds and then decreased with age. In *T. trichiura*, the prevalence of intensity of infections

followed a similar pattern as *A. lumbricoides*. Figures 5-7 show the relationship between the intensity of infection and age for each STH.

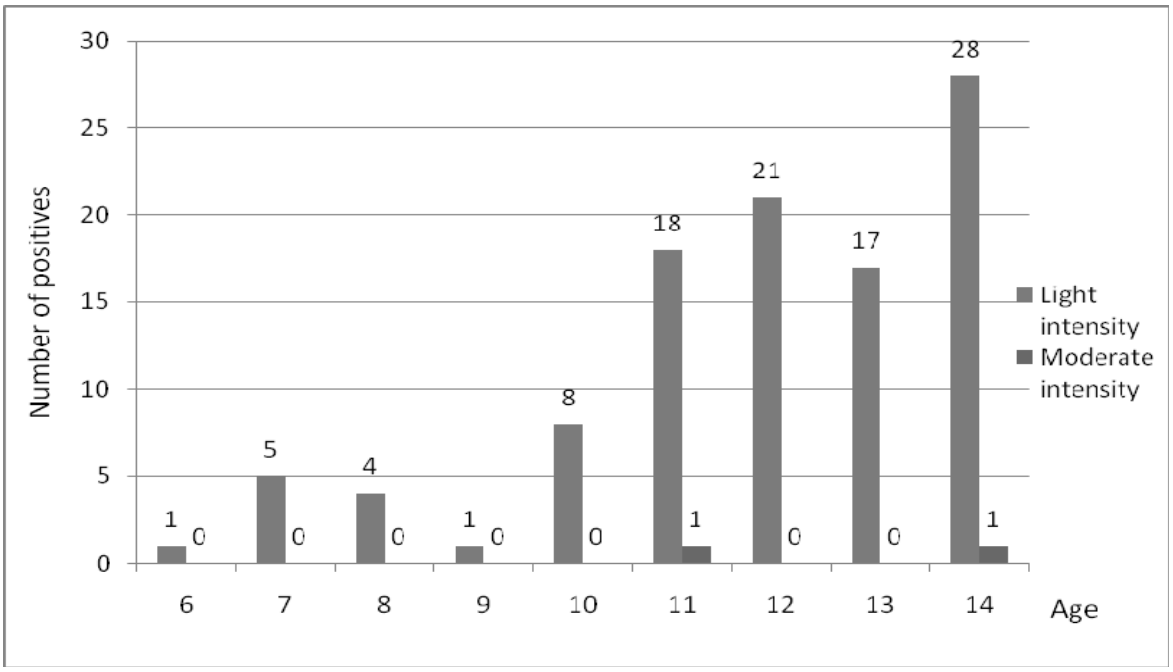

Figure 5: Age wise intensity of infections with hookworms

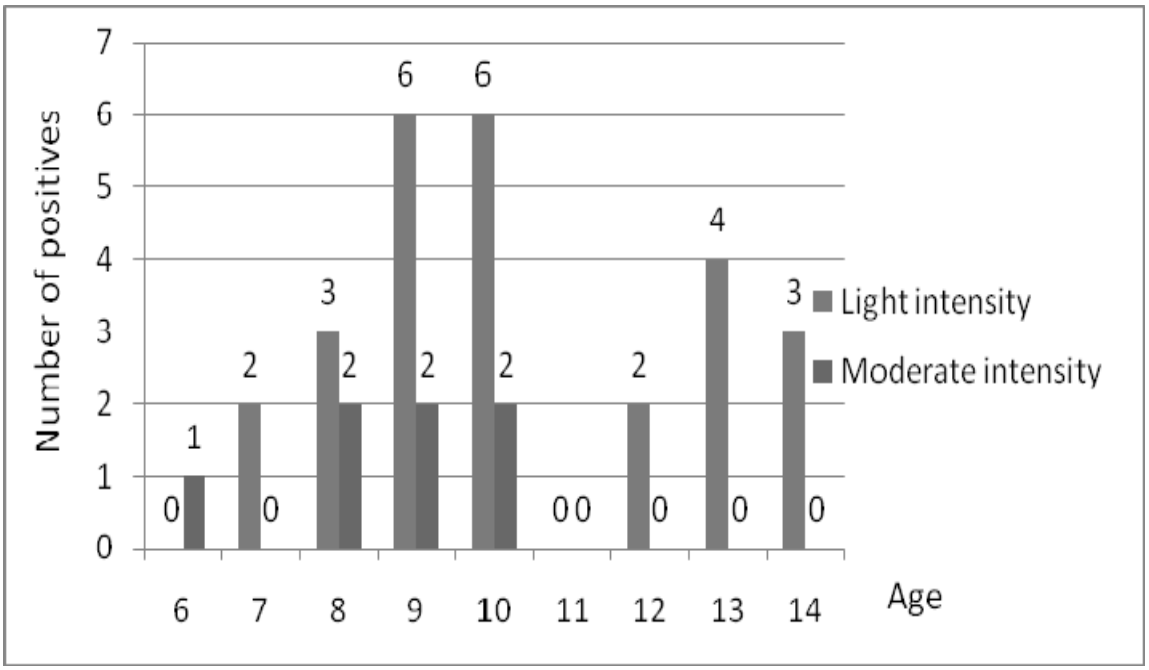

Figure 6: Age wise intensity of infections with *A. lumbricoides*

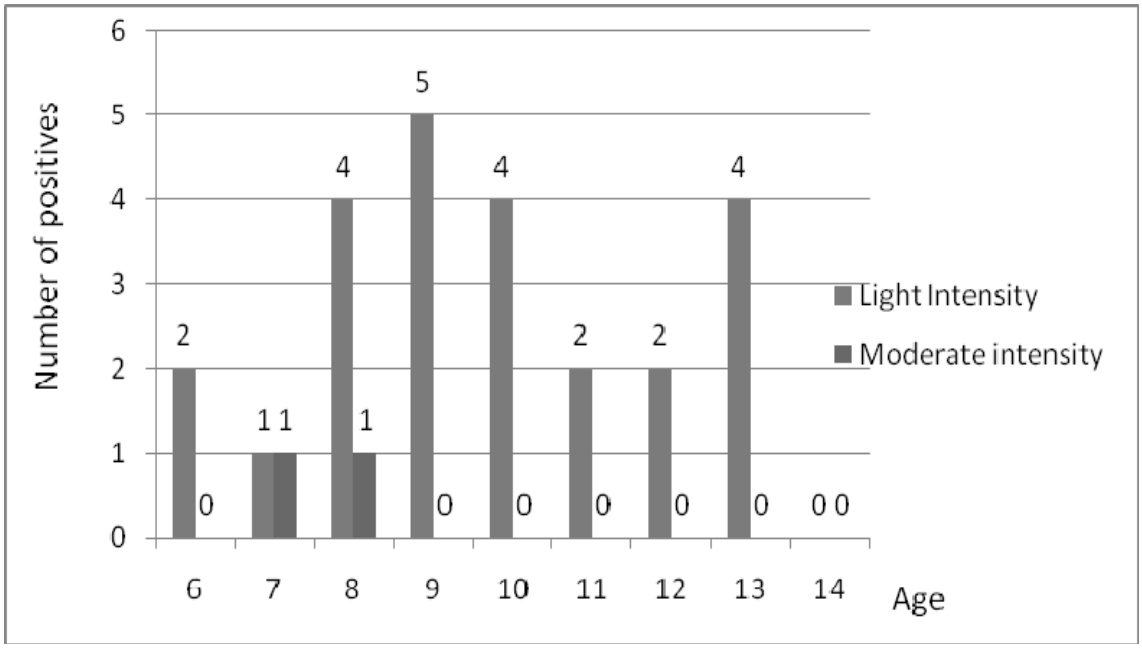

Figure 7: *A*ge wise intensity of infections with *T. trichiura*

Other parasites

In addition to the soil transmitted helminthes, other parasites were also seen on examination of stool specimens. *Giardia* (12.8%) and *Entamoeba histolytica* (1.1%) were common protozoans. Other helminths which were seen were *Enterobius vermicularis* (0.9%), *Hymenolepis nana* (2.3%) and *Strongyloides stercoralis* (0.4%).

3. Efficacy Trial

Among the 115 children infected with hookworm, only 69 had an egg count equal to or greater than 150 epg. Of these 69 children, 49 provided a second stool sample, out of which 10 were from Vellore and 39 from Thiruvannamalai district.

Among the 35 children infected with *A. lumbricoides*, egg count was 150 epg or more in 20 children. 16 out of the 20 children provided a second stool sample. Of these 16 samples, 14 were from Vellore and 2 from Thiruvannamalai district.

In 26 children with *T. trichiura*, 17 had egg counts equal to or greater than 150 epg. Of these 17 children, 13 provided a second stool sample of which 11 were from Vellore and 2 from Thiruvannamalai district.

Cure rate and egg reduction rate

The definitions of cure rate, egg reduction rate, mean change in epg and mean percentage change in epg are given in table 10.

Table 10: Definitions of Cure rate, Egg reduction rate, Mean change in Epg and Mean change in epg

Term	Definition
Cure rate	Ratio of the difference between the number of positive cases before treatment and number of positives after treatment on the number of positives before treatment expressed as percentage
Egg reduction rate	Ratio of the difference between mean epg before treatment and mean epg after treatment on the mean epg before treatment expressed as percentage
Mean change in epg	Mean of change in epg of individual subjects between the pre and post-intervention surveys
Mean percentage change in epg	Mean percentage change in epg of individual subjects between the pre and post-intervention surveys

Hookworms- The overall cure rate for hookworm was found to be 83.6%, with the cure rate in Vellore and Thiruvannamalai being 90% and 82.1% respectively. There was no statistically significant difference between the cure rates (p=0.54) of the two districts.

The mean change in eggs per gram was 544 overall, and 735 and 495 for Vellore and Thiruvannamalai respectively. The mean percentage change in eggs per gram was 94.9% for all 49 samples and 99.4% and 93.9% for Vellore and Thiruvannamalai, respectively.

The overall egg reduction rate for hookworm was calculated as 96%, with the egg reduction rate in Vellore and Thiruvannamalai being 98.6% and 94.2% respectively.

The cure rates, mean change in eggs per gram, mean percentage change in eggs per gram and egg reduction rate for hookworm are given in the table 11.

Table 11: Hookworm- cure and egg reduction rates

	CR	ERR	Mean change in EPG	Mean % change in EPG
Total(n=49)	83.6%	96%	544	94.9
Vellore (n= 10)	90%	98.6%	735	99.4
Thiruvannamalai (n= 39)	82.1%	94.1%	495	93.9

A significant change was observed between the pre-treatment and post-treatment egg counts of hookworm in Vellore (p=0.005) and Thiruvannamalai districts (p=0).

A. lumbricoides - The overall cure rate for *A. lumbricoides* was found to be 100%.

The mean change in eggs per gram was 2956 overall and 3346 and 225 for Vellore and Thiruvannamalai respectively. The mean percentage change in eggs per gram was 100% for all the samples.

The overall egg reduction rate was calculated as 100%, with the egg reduction rate in both districts being 100% each. Table 12 shows the cure rates, mean change in eggs per gram, mean percentage change in eggs per gram and egg reduction rate for *A. lumbricoides*.

Table 12*: A. lumbricoides*-Cure and egg reduction rates

	CR	ERR	Mean change in EPG	Mean % change in EPG
Total (n=16)	100%	100%	2956	100%
Vellore (n= 14)	100%	100%	3346	100%
Thiruvannamalai (n=2)	100%	100%	225	100%

A significant change was observed between the pre-treatment and post-treatment egg counts of *A. lumbricoides* in Vellore (p=0.001) and Thiruvannamalai districts (p=0.2).

T. trichiura- The overall cure rate for *T. trichiura* was found to be 84.6%, with the cure rate in Vellore and Thiruvannamalai being 90.9% and 50% respectively. There was no statistically significant difference between the cure rates (p=0.3) of the two districts.

The mean change in eggs per gram was 354 overall and 359 and 325 for Vellore and Thiruvannamalai respectively. The mean percentage change in eggs per gram was 88.7% overall and 91.7% and 71.8% for Vellore and Thiruvannamalai respectively.

The overall egg reduction rate was 76% which was lower when compared to the other two worms. The egg reduction rate in Vellore and Thiruvannamalai were 79.7% and 59.1% respectively. There was no statistically significant difference between the egg reduction rates (p=0.4) of the two districts.

Table 13: *T. trichiura*-Cure and egg reduction rates

	CR	ERR	Mean change in EPG	Mean % change in EPG
Total(n=13)	84.6%	76 %	354	88.7%
Vellore (n= 11)	90.9%	79.7%	359	91.7%
Thiruvannamalai (n=2)	50%	59.1%	325	71.8%

A significant change was observed between the pre-treatment and post-treatment egg counts of *T. trichiura* in Vellore (p=0.003) and Thiruvannamalai districts (p=0.2).

DISCUSSION

In any field study to assess the efficacy of an anthelminthic in reduction of faecal egg counts, it is important to have techniques that are robust, easy to use and applicable to samples collected in field settings, for a reasonably accurate estimation of the infection intensity based on faecal egg counts (FEC). A recent study comparing different techniques (ether-based concentration, Parasep SF, FLOTAC and McMaster) for the detection of *Trichuris* eggs in non-human primates based on sensitivity, fecal egg counts (FEC), feasibility and ability of estimating drug efficacy indicated that the McMaster technique holds promise for monitoring drug efficacy (Levecke, De Wilde et al. 2009), but this study had not included the Kato-Katz thick-smear (Katz, Chaves et al. 1972). Therefore, this study compared the McMaster with the Kato-Katz thick smear. Both sensitivity and FEC were compared for the two different techniques.

Examining the qualitative data from the two techniques, overall hookworms were the most prevalent by both techniques, but Kato-Katz had slightly higher overall sensitivity (86% compared to 80%, but with overlapping confidence intervals). Both techniques were equally sensitive in samples with high FEC. Examining the quantitative data, McMaster detected significantly more eggs (P=0.016). The most important drawback of the Kato-Katz technique is the diverse clearing time of the different eggs of STH, eggs of hookworms in particular, which impedes further standardization of this technique in large-scaled studies at different study sites (Ramsan, Montresor et al. 1999; Goodman, Haji et al. 2007). Moreover, it is assumed that the templates result in a fixed amount of feces (50mg [diameter (ø) = 9 mm, thickness (t) = 1mm]; 41.7mg [ø = 6 mm, t = 1.5 mm]; 20mg [ø = 6.5 mm, t = 0.5 mm]). But the density of the samples may be affected by various factors, and therefore result in biased estimates of the eggs per gram of feces. This comparative study indicated that the Kato-Katz technique is likely to be less

appropriate for an accurate estimation of FEC and confirms that McMaster holds promise as the method of choice for monitoring drug efficacy.

The pretreatment prevalence of STH in Vellore district (a MDA area which has received several rounds of DEC and albendazole) in this study was found to be 6.8%. Since pre-MDA prevalence in this area is not known, it is not possible to comment on the impact of mass treatment with albendazole on the prevalence, although a higher prevalence (14.15%) of STH was found in a study in the neighboring district of Villupuram after two rounds of annual MDA with a combination of DEC and albendazole (Mani, Rajendran et al. 2004). The lower prevalence in this study could be explained by the fact that this study was conducted after seven rounds of MDA. A study done in Indonesia showed that the prevalence of hookworm in children was found to be 8.2% following annual MDA with DEC and albendazole (Oqueka, Supali et al. 2005). In a study done in Tuvalu, prevalence of hookworm was 1.2% in children aged 5-12 years following annual MDA with DEC and albendazole (Speare, Latasi et al. 2006). However, in the above study the number of children examined was lesser (n=171) than in our study (n=1161).

The prevalence in the non-MDA area (Thiruvannamalai district) was found to be much lower when compared to similar studies done in this region (Kang, Mathew et al. 1998; Paul and Gnanamani 1998; Fernandez, Verghese et al. 2002; Naish, McCarthy et al. 2004). The reason for this is unclear, but could be due to increased awareness of these parasites and increased use of footwear, increased treatment or improved sanitary conditions. Unexpectedly, there was no significant difference between the overall prevalence of STH in the two districts. However, there was a significant difference in the prevalence of individual STH between the districts. The prevalence of hookworm infection was higher in Thiruvannamalai district in which the selected schools were situated in rural areas while the prevalence of *A. lumbricoides* and *T. trichiura* was

higher in Vellore district, in which the selected schools were in urban areas. A similar observation was found in other studies (Albonico, Chwaya et al. 1997; Curtale, Shamy et al. 1998; Fernandez, Verghese et al. 2002). The higher prevalence of *A. lumbricoides* and *T. trichiura* in urban areas could be due to the lack of hygienic water supply, improper sewage disposal and overcrowding. Apart from these factors, eggs of *A. lumbricoides* are hardier and adhere to different environmental surfaces easily. Hookworm transmission mainly occurs following exposure to soil contaminated with hookworm larvae.

The cure rate and egg reduction rate for *A. lumbricoides* in this study was 100%. This is in agreement with previous studies (El-Masry, Trabolsi et al. 1983; Stephenson, Latham et al. 1993; Beach, Streit et al. 1999) and it showed that albendazole at 400 mg was highly effective in treatment of infections with *A. lumbricoides*. The cure rate of 83% and egg reduction rate of 90% for hookworm infections was also in concordance with previous studies (Bartoloni, Guglielmetti et al. 1993; Sacko, De Clercq et al. 1999). A review which summarizes numerous efficacy studies with 400 mg of albendazole for *T. trichiura* infections depicted a median CR of 38% and a median ERR of 80% (Bennett and Guyatt 2000). In this study, CR was markedly higher and ERR was in agreement with this value. It is known that in whipworm infections, a single dose of albendazole is sub curative and a higher dose or multiple doses are required to clear the infection (Adams, Lombard et al. 2004).

There was a significant change in post treatment epg values when compared to the pretreatment epg values for all the three STHs in the MDA area. The egg reduction rates for *A. lumbricoides*, hookworms and *T. trichiura* in the MDA area were 100%, 96% and 76% respectively. A similar significant change was observed in the non MDA areas for all the STHs except *T. trichiura*. These findings suggest that despite several rounds of MDA, STHs can continue to be treated

with albendazole in these areas currently. However, the scale up of chemotherapy programs that are underway in various parts of Africa, Asia and South America, particularly targeting school age children is likely to exert increasing drug pressure on the parasite population.

Given the paucity of suitable alternative anthelminthics, it is imperative that monitoring programs are introduced, both to assess progress and to detect any changes in therapeutic efficacy that may arise from the selection of worms carrying resistant genes. The lack of a widely accepted Standard Operating Procedure (SOP) for undertaking such trials has been a felt need, because published studies are confounded by methodological variations including treatment regimens, sources of drugs, statistical analyses used to calculate therapeutic efficacy, as well as inherent problems such as insufficient sample size, diagnostic tests with variable test characteristics, variation in baseline infection intensities and confounding factors related to geographical locations. Such variation among studies greatly complicates direct comparison. A WHO -WB meeting on "Monitoring of Drug Efficacy in Large Scale Treatment Programmes for Human Helminthiasis", held in Washington DC at the end of 2007, highlighted the need to closely monitor anthelminthic drug efficacy and to develop SOPs for this purpose. In a systematic meta-analysis of published single-dose studies, Keiser and Utzinger confirmed that there was a paucity of high quality trials, and that the majority of trials were carried out more than 20 years ago. They recommended that well- designed, adequately powered, and rigorously implemented trials should be undertaken to provide current data regarding the efficacy of anthelminthics against the main species of STH. These should be designed to establish benchmarks (including SOPs) for subsequent monitoring of drug resistance. The objective of the present work was to validate a standard protocol that has been developed for monitoring efficacy of anthelminthics against STH. We assessed the efficacy of a single dose 400 mg of ALB in

terms of the CR and ERR in school age children following treatment. The McMaster egg counting technique was used in a standardized fashion, with rigorous quality control.

Limitations

A placebo group was not included to compare with the treatment groups in both the areas in order to determine the efficacy of albendazole, but this was because it would be unethical to use a placebo when effective treatment exists. The numbers of children infected with each of the STH were unequal in the MDA and non MDA areas. In future studies, it may be possible to base the treatment comparison on cases found to be infected with specific STHs, but for the lower prevalence worms, such as *Trichuris*, this would require the screening of several thousand additional specimens.

SUMMARY

1. In the pilot study, the McMaster's method had a slightly lower sensitivity when compared to the Kato-Katz method in the detection of the STHs. However, the McMaster's method was found to be a simple and more appropriate technique for an accurate estimation of FEC.
2. The prevalence of STH in Vellore District and Thiruvannamalai district was found to be 6.8% and 7.1% respectively. There was no significant difference between the prevalence of STH between the two districts, but the prevalence of individual STH worms varied significantly between the two districts.
3. The cure rate of hookworms, *T. trichiura* and *A. lumbricoides* was found to be 83.6%, 84.6% and 100% respectively. The egg reduction rates for hookworms, *T. trichiura* and *A. lumbricoides* were 96%, 76% and 100% respectively.
4. A significant change was found between the pre-treatment and post-treatment fecal egg counts of all the three STHs in both the districts, with no indication of a difference in response to treatment with albendazole in the two districts.

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ANNEXURE

PATIENT CONSENT FORM

Title of Clinical Trial: Efficacy of Albendazole in reducing fecal egg counts in previously treated school children.

This is a clinical trial to test whether a drug (Albendazole) which is used for infection with soil transmitted helminths is as effective in people who live in areas where the government has been using it for a long time as in people who live where the drug has not been used as part of a public health program.

I have /my child has been selected to participate in this study because I have/my child has an infection with *A. lumbricoides, T. trichiura* and hookworm.

If my child participate(s):

1. My child will be given one tablet of Albendazole for treatment
2. My child will be part of this study from treatment for a period of 10 days until collection of one additional stool sample.
3. The tests being done on the stool samples are to identify how much infection with soil transmitted helminths is present and whether the treatment cured or reduced the infection.
4. The results from this study will help decide whether or not the medicine that I am/my child is being given helps to removing or reducing the infection with soil transmitted helminths in places where albendazole has been used in the government program for filarial (elephantiasis) treatment
5. If the tests show that the treatment with Albendazole did not work, then my child will be given treatment again until my child is cured.
6. I will not have to pay for any of the stool tests or deworming medicines. My child and I will not receive free treatment for any other illness.
7. I give my consent to the samples collected for this study to be stored and tested in the future for other pathogens and markers of infection.
8. No foreseeable harm or discomfort is expected to arise from participating in this study.
9. My consent for my child to be included in this study is purely voluntary. I may withdraw from it at any time without any penalty. If I do not wish to continue in the study, my child can continue to receive treatment for any illnesses either in this hospital, or in any other hospital.
10. My/his/her hospital records will remain confidential.
11. I may contact Dr. Vipin Sam Alexander(0416-228-2588) if I have any further questions about the research. I may contact Dr. Lionel Gnanaraj, the Medical Superintendent (0416-228-2006), or the Office of Research at the Christian Medical College (0416-228-4202) if I feel that the treatment given caused adverse effects.

I, _____, father/mother of_____ ,

Hosp. No._____. /Study No._____, do hereby consent for my child to be enrolled for this study. The nature and purpose of the trial has been explained to me by _____.

Signature of Parent/Guardian: Name: Date:

Signature of the witness: Name: Date:

I hereby certify that I have explained the above to the participant/Parent/Guardian.

Name of Study Staff: Date:

Signature of Study Staff: